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(54) Title: SYNERGISTIC BIOCIDAL COMBINATIONS

(57) Abstract

The present invention is directed to microbiocidal combinations and processes for inhibiting the growth of microorganisms. The novel combinations and processes of the present invention show unexpected activity against microorganisms, including bacteria, fungi and algae. Specifically, the microbiocidal combinations comprise (i) an oxidant or oxidizing biocide such as potassium monopersulfate, sodium perborate, hydrogen peroxide or sodium percarbonate, (ii) a non-oxidizing microbiocide such as glutaraldehyde and optionally (iii) a surfactant/dispersant, (iv) an anti-corrosive material, and (v) an anti-scale material.

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SYNERGISTIC BIOCIDAL COMBINATIONS

FIELD OF THE INVENTION

The present invention relates to microbiocidal compositions and to processes of utilizing these microbiocidal compositions for inhibiting the growth of microorganisms in aqueous systems. More particularly, the microbiocidal compositions of this invention comprise a combination of (i) an oxidant, and (ii) a non-oxidizing microbiocide optionally with a surfactant/dispersant, an anti-corrosive material, and/or an anti-scale material.

BACKGROUND OF THE INVENTION

The proliferation of microorganisms and resultant formation of slime is a problem which commonly occurs in many aqueous systems. Problematic slime producing microorganisms include bacteria, airborne microorganisms, sulfate reducing bacteria, fungi and algae. Slime deposits commonly form in many industrial aqueous systems including cooling water systems, pulp and paper mill systems, petroleum operations, industrial lubricants, cutting fluids, coolants, etc. The formation of slime by microorganisms in these systems is a significant and constant problem.

For example, slime deposits deteriorate cooling towers made of wood and promote corrosion when deposited on the metal surfaces of cooling water systems. Furthermore, slime deposits tend to plug or foul pipes and valves and reduce heat exchange or cooling efficiency on heat exchange surfaces.

Pulp and paper mill systems operate under conditions which encourage the growth of microorganisms and often results in fouling problems. Moreover, microorganisms can form large slime deposits which can become dislodged and show up in the paper product as spots, holes or tears. This necessitates shutting down the paper making

process to clean the equipment, and results in lost production time.

Slime may also be objectionable from the standpoint of cleanliness and sanitation in breweries, wineries, dairies and other industrial food and beverage process water systems. Moreover, sulfate reducing bacteria are often problematic in waters used for the secondary recovery of petroleum or for oil drilling in general. For example, these organisms reduce sulfates present in the injection water to form insoluble iron sulfide deposits and may enhance corrosion of metals by accelerating galvanic action.

The proliferation of bacteriological contamination in lubricants and cutting fluids is a common problem due to the elevated temperature and unsanitary conditions found in many metal working plants. It is often necessary to discard these fluids due to microbiological contamination.

Accordingly, because of the foregoing problems in various industrial processes, numerous biocidal materials have been employed to eliminate, inhibit or to reduce microbial growth. Various oxidizing biocides have enjoyed widespread use in such applications including chlorine, chlorine dioxide and bromine. However, these oxidizing biocides are not always effective for controlling microbiological growth. For example, oxidizing biocides are consumed by inorganic species such as ferrous iron, reduced manganese, sulfides, etc. as well as organic compounds which are commonly found in those systems.

In addition, the effectiveness of a biocide is rapidly reduced as a result of exposure to adverse physical conditions such as temperature or contact with incompatible water treating agents in the system.

Therefore multiple doses or large quantities of expensive biocidal chemicals have heretofore been required in order to maintain control over microbial growth.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide novel microbiocidal compositions which provide enhanced effectiveness for controlling or inhibiting the growth of microorganisms in an aqueous system.

It is another object of this invention to provide an improved process for controlling microorganisms in aqueous systems such as pulp and paper mill systems, cooling water systems, metal working fluids and petroleum operations.

It is another object of this invention to reduce the level of toxic biocides in industrial-water effluents. It is an advantage of the present invention that the biocidal compositions permit a reduction in the dosage amount of biocide required to treat nuisance microbiota in industrial waters, and significantly reduces the time required to control microbiological organisms.

In accordance with the present invention, there have been provided certain novel biocidal compositions which are used to control or inhibit microbial growth, comprising (i) a microbiocidal effective amount of an oxidant selected from the group of mono- or di-peroxyorganic acids, halogen dioxides, monopersulfates, halogens, halogen releasing compounds, perborates, peroxides, persulfates, permanganates, percarbonates, ozone, and water soluble salts thereof, and mixtures thereof; and (ii) a microbiocidal effective amount of a non-oxidizing microbiocide selected from the group consisting of glutaraldehyde, limonene, bis(trichloromethyl) sulfone, 2-(decylthio)-ethanamine, dodecylguanidine hydrochloride, 2-(2-bromo-2-nitroethyl)

furan, poly(oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride), alkyl dimethyl benzyl ammonium chloride, alkylamidopropyl propylene glycol dimethyl ammonium chloride phosphate, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, tetrakis-hydroxylmethyl phosphonium sulfate, tributyltetradecyl phosphonium chloride, 2-bromo-2-nitropropane-1, 3-diol, and 2,2-dibromo-2-nitroethanol and sanguinaria extract.

Also provided in accordance with the present invention is a method for controlling or inhibiting microbial growth in aqueous systems comprising adding to the system (i) a microbiocidal effective amount of an oxidant selected from the group of mono- or di-peroxyorganic acids, halogen dioxides, monopersulfates, halogens, halogen releasing compounds, perborates, peroxides, persulfates, permanganates, percarbonates, ozone, their water soluble salts, and mixtures thereof; and (ii) a microbiocidal effective amount of a non-oxidizing microbiocide selected from the group consisting of glutaraldehyde, limonene, bis(trichloromethyl) sulfone, 2-(decylthio)-ethanamine, dodecylguanidine hydrochloride, 2-(2-bromo-2-nitroethyl) furan, poly(oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride), alkyl dimethyl benzyl ammonium chloride, alkylamidopropyl propylene glycol dimethyl ammonium chloride phosphate, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, tetrakis-hydroxylmethyl phosphonium sulfate, tributyltetradecyl phosphonium chloride, 2-bromo-2-nitropropane-1, 3-diol, 2,2-dibromo-2-nitroethanol and sanguinaria extract.

DETAILED DESCRIPTION

The present invention is directed to certain novel biocidal compositions comprising combinations of oxidants

and non-oxidizing biocides which are added to an aqueous system in amounts effective to inhibit or control the growth of microorganisms in the aqueous system. More particularly, the biocidal compositions of this invention comprise combinations of (i) an oxidant selected from the group consisting of mono- or di-peroxyorganic acids, halogen dioxides, monopersulfates, halogens, halogen releasing compounds, perborates, peroxides, persulfates, permanganates, percarbonates, ozone, their water soluble salts, and mixtures thereof and (ii) a non-oxidizing microbiocide selected from the group consisting of glutaraldehyde, limonene, bis(trichloromethyl) sulfone, 2-(decylthio)ethanamine, dodecylguanidine hydrochloride, 2-(2-bromo-2-nitroethyl) furan, poly(oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride), alkyl dimethyl benzyl ammonium chloride, alkylamidopropyl propylene glycol dimethyl ammonium chloride phosphate, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, tetrakis-hydroxylmethyl phosphonium sulfate, tributyltetradecyl phosphonium chloride, 2-bromo-2-nitropropane-1, 3-diol, 2,2-dibromo-2-nitroethanol, sanguinaria extract and optionally in combination with (iii) a surfactant/ dispersant, (iv) an anti-corrosive agent and (v) an anti-scale agent. The above oxidants and non-oxidizing microbiocides of this invention are commercially available or may be easily synthesized from commercially available raw materials by known methods.

Suitable peroxides include inorganic peroxides such as hydrogen peroxide, sodium peroxide, as well as organic peroxides such as benzoyl peroxide and the like. Suitable halogen releasing compounds include hydantoins such as 1,3-dichloro-5,5-dimethyl hydantoin, 1,3-dibromo-5,5-dimethyl hydantoin or 1,3-diiodo-5,5-dimethyl hydantoin. Suitable mono- or di-peroxyorganic acids

include, but are not limited to, peracetic acid, perbenzoic acid, peroxypropionic acid, hexane diperoxoic acid, dodecanediperoxoic acid. Suitable halogen dioxides include chlorine dioxide, bromine dioxide and iodine dioxide. Specific examples of other suitable oxidants include sodium perborate, sodium percarbonate, potassium permanganate, sodium persulfate, potassium persulfate, ammonium persulfate, chlorine, bromine, iodine and chlorine, bromine, iodine releasing compounds, sodium monopersulfate, potassium monopersulfate, and ammonium monopercarbonate. Potassium monopersulfate is a preferred oxidant and is commercially available from DuPont as OXONE.

The combination of the above oxidants and non-oxidizing biocides unexpectantly provide enhanced biocidal activity which is greater than that of the individual components which make up the mixtures. The microbiocidal compositions of the present invention possess a high degree of slimicidal activity which could not have been predicted from the known activities of the individual ingredients comprising the combination. The enhanced activity of the mixture permits a significant reduction in the total quantity of the biocide required for an effective treatment of an aqueous system. The enhanced biocidal effectiveness of the compositions of the present invention was particularly surprising since not all oxidants provide enhanced biocidal activity when used in combination with non-oxidizing biocides. In fact, some oxidants are actually antagonistic when used in combination with non-oxidizing biocides, and result in less biocidal effectiveness than the use of either component alone.

The biocidal combinations of this invention are effective for controlling and inhibiting the growth and

reproduction of microorganisms in cooling water systems, pulp and paper mill systems, petroleum operations (e.g. oil well applications), industrial lubricants and coolants, lagoons, lakes and ponds, etc. The particular type of microorganisms present in these areas vary from location to location, and even at a given location over a period of time. Representative examples of microorganisms which may effectively be treated with the biocidal compositions of the present invention include fungi, bacteria and algae and, more particularly, include such genera as Aspergillus, Penicillium, Candida, Saccharomyces, Aerobacter, Escherichia, Alcaligenes, Bacillus, Chlorella, Spirogyra, Oscillatoria, Vaucheria, Pseudomonas, Salmonella, Staphylococcus, Pullularia, Flavobacterium and Rhizopus.

In accordance with the invention, an aqueous system is treated to inhibit the growth of the microorganisms by adding to the aqueous system at least one oxidant and at least one non-oxidizing microbiocide. These components are present in the system at the same time. While it is possible to combine the oxidant and the non-oxidizing biocide, it is generally preferred not to combine the microbiocide with the oxidant too far in advance of being added to the aqueous system because these materials may adversely react when they are brought into direct contact with each other in their concentrated forms.

The dosage amounts of oxidant and non-oxidizing biocide which are added to an aqueous system may vary widely depending upon the nature of the aqueous system being treated, the level of organisms present in the aqueous system and the level of inhibition desired. An important consideration when dosing the oxidants of the present invention are the levels of ferrous iron, reduced manganese, sulfide, ammonia, organic constituents, and

the like, which may react with and thereby consume the oxidants of the present invention. "Oxidant Demand" refers to the difference between the oxidant dosage amount and the residual oxidant concentration after a prescribed contact time and at a given pH and temperature. "Oxidant Requirement" refers to the oxidant dosage amount required to achieve a given residual oxidant concentration at a prescribed contact time, pH and temperature. Since the levels of ferrous iron, reduced manganese, sulfide, etc. can vary widely from system to system, the oxidant demand should be determined for the aqueous system being treated in accordance with the method of this invention. For purposes of this invention, the dosage amount of oxidant which is added to an aqueous system, i.e., a biocidally effective amount, refers to the residual oxidant concentration in an aqueous system. Residual oxidant concentration can readily be determined by one skilled in the art by conventional means.

In general, the dosage amount of oxidant may be from 0.1 ppm to 100 ppm, preferably from about 0.5 ppm to about 45 ppm. The dosage amount of microbiocide in the system may be from 0.1 ppm to 125 ppm, preferably about 0.5 ppm to about 45 ppm. When the microbiocides and oxidants are present in the above amounts, the resulting combination possesses a higher degree of effectiveness against microorganisms than the individual components comprising the combination. While larger quantities of the microbiocides or oxidant may be used with no detrimental effect, such large quantities increase the cost of treatment and generally provide little additional benefit.

The biocidal compositions of the present invention may optionally be used in combination with one or more

surfactants/dispersants to disperse biomass and to enhance the dispersibility and stability of these microbiocidal formulations. Suitable surfactants/ dispersants include, but are not limited to, cationic, nonionic, anionic, or amphoteric surfactants and polymers such as fluorinated surfactants, alkylaryl polyether alcohols, polyether alcohols, sodium dodecyl sulfate, sodium nonylbenzene sulfonate, sodium dioctyl sulfosuccinate, octylphenoxypolyethoxyethanol, ethylene and/or propylene oxide condensates with long chained alcohols, mercaptans, amines, carboxylic acids, sodium sulfonate of condensated naphthalene-formaldehyde and lignin sulfonate, alkyl benzene sulfonates and sulfates, sodium linear dodecyl benzene sulfonate, propylene oxide-ethylene oxide block copolymers such as, e.g., a polyoxypropylene glycol polymer having a molecular weight of from 1500-2000 which has been reacted with from 5-30% by weight of ethylene oxide (commercially available from BASF as the Pluronic and Tetronic surfactants), and the like. Preferred fluorinated surfactants include those manufactured by 3M such as FC-99, FC-100 and FC-129. FC-99 is an anionic surfactant which is a 25% active solution of amine-perfluoroalkyl sulfonates in water. FC-100 is an amphoteric surfactant which is a 28% active solution of fluorosurfactant solids in glycol/water. FC-129 is an anionic surfactant which is a 50% solution of potassium fluorinated alkyl carboxylates in water, butyl cellosolve and ethanol. The dosage amount of surfactant/dispersant in the aqueous system is not critical, per se, provided of course that it is added in an amount effective to disperse the biomass or stabilize a particular microbiocidal formulation. These dosage amounts are typically from 0.5 to 500 ppm.

The biocidal compositions of the present invention may also be used in combination with an anti-corrosive material. Suitable anti-corrosive materials include, but are not limited to, phosphates such as sodium tripolyphosphate or tetrapotassium pyrophosphate, phosphonates, carboxylates, etc. These components may be added to help protect mild steel from corrosive attack by the oxidant. The anti-corrosive material may be blended with the oxidant before being added to the system or may be added separately. The anti-corrosive material is generally added to the system in a dosage amount of from 0.5 to 50% based on the total amount of oxidant and anti-corrosive material in the mixture. More preferably, the amount of the anti-corrosive material is at least 1% of the total amount of oxidant and anti-corrosive material in the mixture.

The biocidal compositions of this invention may also be used in combination with other biocides which further enhance the synergistic effectiveness. For example, preferred biocidal combinations include glutaraldehyde with isothiazolone. The ratio of these biocidal combinations can range from 1:10 to 10:1 on a weight basis. A preferred isothiazolone is a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one.

The biocidal compositions of the present invention may also be used in combination with an anti-scale material. Suitable anti-scale materials include, but are not limited to, polyacrylates such as sodium polyacrylate, phosphonates such as hydroxyethylidene diphosphonic acid, etc. The anti-scale material is generally added to the system in a dosage amount of from 0.5 to 50% based on the total amount of oxidant and anti-scale material in the mixture.

The oxidants of this invention may be in solid or liquid form and may be diluted with a solid or liquid carrier. Powders may be prepared with a finely divided solid carriers including talc, clay, pyrophyllite, diatomaceous earth, hydrated silica, calcium silicate, or magnesium carbonate. Powders may typically contain 1 to 15 percent of the microbiocides of this invention, while a wettable powder may be obtained by increasing the proportion of microbiocide to about 50 percent or more. A typical formulation of a wettable powder comprises 20 percent to 50 percent of the suitable compounds of this invention, 45 percent to 75 percent of one or more finely divided solids, one percent to five percent of a wetting agent, and one percent to five percent of a dispersing agent.

The oxidants of this invention may also be used in the form of liquid concentrates. These are prepared by diluting or dissolving the oxidants and/or microbiocides of this invention in a solvent together with one or more surface active agents.

The following examples are provided to illustrate the present invention in accordance with the principles of this invention, but are not to be construed as limiting the invention in any way except as indicated in the appended claims. All parts and percentages are by weight unless otherwise indicated.

The synergism of the two-component microbiocidal combinations of the present invention was demonstrated by testing a wide range of concentrations and ratios of compounds, generated by two-fold serial dilutions in a liquid. The liquid medium is composed of deionized water supplemented with inorganic constituents to simulate an industrial water. Work was performed with the bacterium *Enterobacter aerogenes* or a mixed bacteria culture

consisting of *Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtilis;* the fungus *Aspergillus* niger; and for the algae, Chlorella vulgaris, or *Scenedesmus quadracauda*. All organisms were representative of those typically found in industrial waters. Contact periods ranged from 2-24 hours and incubations of subcultures to agar surfaces were conducted at temperatures, times, and lighting conditions permitting the growth of visible colonies. For bacteria the agar was Tryptic Soy, for algae CHU-10, and for fungi Potato Dextrose Yeast Extract. Uniform inoculations within tests of simulated industrial waters were made such that all test-solutions and all exposures of organisms were made with the same density of organisms per ml. After inoculation of the simulated industrial waters these cell-densities were in the thousands per ml for fungi-spores, and algae, and in the millions per ml for bacteria. Following the bacteria-biocide contact in liquid medium and subculture to agar, organic nutrients in the form of sterile Tryptic Soy Broth was added to each tube or well of the bioassay followed by reincubation to determine the viability of any surviving organisms expressed as turbidity (growth). No-growth of bacteria resulted in clear medium without turbidity.

Various end-points of colony formation were employed for the calculation of synergies from 90 percent to 100 percent reduction as compared to untreated controls.

The test results for demonstration of synergism of biocide combinations are illustrated in the following examples.

Each table in the examples is organized to show synergy by illustrating (1) the concentration of each test material acting alone required to produce a given end-point of growth prevention or colony-forming-unit

inhibition as compared to untreated controls; and (2) the lower required concentrations of the combined test materials.

Example 1

This example shows synergies between glutaraldehyde and H_2O_2 using the bacterium *Enterobacter aerogenes* and a mixed bacteria culture.

Table 1

Active Biocide Concentrations to Achieve 100% Inhibition of *Enterobacter aerogenes*, 4 Hours Contact

Glutaraldehyde, Mg/l	H_2O_2, Mg/l
64	0
0	200
4	6.3

Table 2

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

Glutaraldehyde, Mg/l	H_2O_2, Mg/l
100	0
0	100
3.8	50
7.5	25
15	3.1

Example 2

This example demonstrates synergies between glutaraldehyde and H_2O_2 using the green algae *Chlorella vulgaris* and *Scenedesmus quadracauda*.

Table 3

Active Biocide Concentrations to Achieve 100% Inhibition of *Chlorella vulgaris* plus Unknown Ancillary Bacteria 23 Hours Contact

Glutaraldehyde, Mg/l	H_2O_2, Mg/l
>320	0
0	40
5	20

Table 4

Active Biocidal Concentrations to Achieve 100% Inhibition of *Scenedesmus quadracauda* 4 Hours Contact

Glutaraldehyde, Mg/l	H_2O_2, Mg/l
80	0
0	40
5	5

Example 3

The data (Table 5) indicate hydrogen peroxide as synergist with a combination of glutaraldehyde and Kathon 886F(4:1 active) using the green algae *Chlorella vulgaris*. Kathon 886F is a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one.

Table 5

Active Biocide Concentrations to Achieve 100% Inhibition of *Chlorella vulgaris* 2 Hours Contact

Glutaraldehyde/Kathon, Mg/l	H_2O_2, Mg/l
416	0
0	100
104	6.3
52	12.5

Example 4

This example demonstrates synergies between limonene and oxidizing biocides using a mixture of four bacteria species.

Table 6

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

d-Limonene, Mg/l	H_2O_2, Mg/l	Peracetic Acid, Mg/l
>2,000	0	0
0	300	0
0	0	23.5
31	75	0
31	0	11.8

Example 5

This example demonstrates synergies between 2-(decylthio)-ethanamine (DTEA) and the oxidizing biocide hydrogen peroxide employing a mixed bacteria culture (Table 7).

Table 7

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

DTEA, Mg/l	H_2O_2, Mg/l
100	0
0	100
25	3.1

Example 6

This example demonstrates synergies between 2,2-dibromo-2-nitroethanol and the oxidizing biocide hydrogen peroxide.

Table 8

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

2,2-Dibromo-2-nitroethanol, Mg/l	H_2O_2, Mg/l
7.8	0
0	>200
3.9	3.1

Example 7

This example demonstrates synergies between poly(oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride) (WSCP) and the oxidizing biocide hydrogen peroxide. In this test the bacterium *Enterobacter aerogenes* was employed as the test organism.

Table 9

Active Biocide Concentrations to Achieve 100% Inhibition of *Enterobacter aerogenes* 4 Hours Contact

WSCP, Mg/l	H_2O_2, Mg/l
2	0
0	30
1	5

Example 8

This example demonstrates synergies between tetradecyl dimethyl benzyl ammonium chloride and the oxidizing biocide hydrogen peroxide.

Table 10

Active Biocide Concentrations to Achieve 100% Inhibition of *Enterobacter aerogenes* 4 Hours Contact

Tetradecyl Dimethyl Benzyl Ammonium Chloride, Mg/l	H_2O_2, Mg/l
20	0
0	100
2.5	20
5	10

Example 9

This example demonstrates synergy between tetrakis-hydroxylmethyl phosphonium sulfate and hydrogen peroxide (Table 11).

Table 11

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

Tetrakis-Hydroxylmethyl Phosphonium Sulfate, Mg/l	H_2O_2, Mg/l
31.2	0
0	100
15.6	50

Example 10

This example shows synergy between tributyltetradecyl phosphonium chloride and hydrogen peroxide (Table 12).

Table 12

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

Tributyltetradecyl Phosphonium Chloride, Mg/l	H_2O_2 Mg/l
31.3	0
0	200
7.8	3.1

Example 11

This example demonstrates synergies between 2-bromo-2-nitropropane-1,3-diol and hydrogen peroxide (Table 13).

Table 13

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

2-Bromo-2-Nitropropane-1,3-Diol, Mg/l	H_2O_2, Mg/l
62.5	0
0	100
7.8	25
15.6	6.3

Example 12

This example demonstrates synergies between cocamidopropyl propylene glycol dimethyl ammonium chloride phosphate and hydrogen peroxide (Table 14).

Table 14

Active Biocide Concentrations to Achieve 100% Inhibition of *Enterobacter aerogenes* 2 Hours Contact

Cocamidopropyl Propylene Glycol Dimethyl Ammonium Chloride Phosphate Mg/l	H_2O_2 Mg/l
78	0
0	200
39	2.5

Example 13

This example demonstrates synergies between 2,4,4'-trichloro-2'-hydroxydiphenyl ether and hydrogen peroxide (Table 15).

Table 15

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

2,4,4'-Trichloro-2'-Hydroxydiphenyl Ether, Mg/l	H_2O_2 Mg/l
500	0
0	150
31.2	75

Example 14

The data (Table 16) show hydrogen peroxide as a synergist with sanguinaria extract against a mixed bacteria culture.

Table 16

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

Sanguinaria Extract, Mg/l	H_2O_2, Mg/l
31.2	0
0	200
3.9	25
7.8	3.1

Example 15

This example demonstrates synergies between poly(oxyethylene(dimethyliminio) ethylene(dimethyliminio) ethylene dichloride) (WSCP) and potassium monopersulfate.

Table 17

Active Biocide Concentrations to Achieve 99.93% Inhibition of Four Mixed Bacteria Species, 15 Hours Contact

WSCP, Mg/l	Potassium Monopersulfate, Mg/l
16	0
0	>4
8	0.5

Example 16

This example shows synergies between glutaraldehyde and sodium hypochlorite.

Table 18

Active Biocide Concentrations to Achieve 100% Inhibition of Four Mixed Bacteria Species, 4 Hours Contact

Glutaraldehyde, Mg/l	Sodium Hypochlorite, Mg/l as Cl_2
25	0
0	>1
12.5	0.016

Example 17

This example demonstrates synergies between glutaraldehyde and bromine.

Table 19

Active Biocide Concentrations to Achieve 100% Inhibition
of Four Mixed Bacteria Species,
2 Hours Contact

Glutaraldehyde, Mg/l	Bromine, Mg/l
50	0
0	1
3.1	0.125

Example 18

The experimental results (Table 20) indicate peracetic acid as a synergist with glutaraldehyde using a mixed bacteria culture.

Table 20

Active Biocide Concentrations to Achieve 100% Inhibition
of Four Mixed Bacteria Species,
4 Hours Contact

Glutaraldehyde, Mg/l	Peracetic Acid, Mg/l
50	0
0	25
6.3	6.3

Example 19

Additional combinations showing synergistic microbiocidal activity are as follows:

(1) Bis(trichloromethyl) sulfone and potassium percarbonate.

(2) Dodecylguanidine hydrochloride and sodium perborate.

(3) 2-(2-Bromo-2-nitroethyl) furan and ozone.

(4) Glutaraldehyde and potassium permanganate.

(5) Glutaraldehyde and chlorine dioxide.

(6) Tributyltetraderyl phosphonium chloride and sodium persulfate.

(7) Bis(trichloromethyl) sulfone and diperoxydodecanoic acid.

(8) d-Limonene, hydrogen peroxide and peracetic acid.

(9) Glutaraldehyde, hydrogen peroxide and sodium nonylbenzene sulfonate.

(10) Glutaraldehyde, peracetic acid and a polyoxypropylene glycol polymer having a molecular weight of 1,650 which has been reacted with 25% by weight of ethylene oxide (Commercially available from BASF as Pluronic L62).

(11) d-Limonene, hydrogen peroxide and sodium dioctyl sulfosuccinate.

<u>Example 20</u>

In this experiment 4,5-dichloro-1,2-dithiol-3-one(Dithiol) was evaluated under the experimental conditions and found that no synergy existed in inhibition between Dithiol and hydrogen peroxide. The results (Table 21) demonstrated no synergy, but a very strong antagonism between the two test-materials, indicating that not all microbiocides may be combined with an oxidant for controlling the growth and deposition of slime forming microorganisms in water.

Table 21

Colony-Forming-Unit Survival on Tryptic Soy Agar after 4-Hour Exposure to Combinations of H_2O_2 and Dithiol

Four Mixed Bacteria Species

Treatment	Number of Colony-Forming-Unit Survival
Dithiol 15.6 ppm	0
Dithiol 15.6 ppm + H_2O_2 25 ppm	$>2 \times 10^6$
Dithiol 62.5 ppm + H_2O_2 25 ppm	$>2 \times 10^6$
H_2O_2 25 ppm	$>2 \times 10^6$

IN THE CLAIMS:

1. A microbiocidal composition comprising:

(i) a microbiocidally effective amount of an oxidant for inhibiting the growth of microorganisms selected from the group consisting of mono- and di-peroxyorganic acids, halogen dioxides, monopersulfates, halogens, halogen releasing compounds, perborates, peroxides, percarbonates, persulfates, permanganates, ozone, and mixtures thereof; and

(ii) a microbiocidally effective amount of microbiocide for inhibiting the growth of microorganisms selected from the group consisting of glutaraldehyde, limonene, bis(trichloromethyl) sulfone, 2-(decylthio)-ethanamine, dodecylguanidine hydrochloride, 2-(2-bromo-2-nitroethyl) furan, poly(oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride), alkyl dimethyl benzyl ammonium chloride, alkylamidopropyl propylene glycol dimethyl ammonium chloride phosphate, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, tetrakis-hydroxylmethyl phosphonium sulfate, tributyltetradecyl phosphonium chloride, 2-bromo-2-nitropropane-1,3-diol, 2,2-dibromo-2-nitroethanol, and sanguinaria extract.

2. A method of inhibiting or controlling the growth and deposition of slime-forming organisms in aqueous systems which comprises adding to the aqueous system a microbiocidal combination comprising:

(i) a microbiocidally effective amount of an oxidant for inhibiting the growth of microorganisms selected from the group consisting of monopersulfates, halogens, halogen releasing compounds, perborates, peroxides, percarbonates, perorganic acid, chlorine dioxide, persulfates, permanganates, ozone, and mixtures thereof; and

(ii) a microbiocidally effective amount of microbiocide for inhibiting the growth of microorganisms selected from

the group consisting of glutaraldehyde, limonene, bis(trichloromethyl) sulfone, 2-(decylthio)-ethanamine, dodecylguanidine hydrochloride, 2-(2-bromo-2-nitroethyl) furan, poly(oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride), alkyl dimethyl benzyl ammonium chloride, alkylamidopropyl propylene glycol dimethyl ammonium chloride phosphate, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, tetrakis-hydroxylmethyl phosphonium sulfate, tributyltetradecyl phosphonium chloride, 2-bromo-2-nitropropane-1,3-diol, 2,2-dibromo-2-nitroethanol, and sanguinaria extract.

3. A method according to Claim 2 wherein the aqueous system is a pulp and paper mill water system.

4. A method according to Claim 2 wherein the microorganisms are sulfate reducing bacteria and wherein the aqueous system is a petroleum operation system.

5. A method according to Claim 2 wherein the microorganisms comprise algae, bacteria, and fungi.

6. A method according to Claim 2 wherein the microorganisms are aerobic bacteria.

7. A method according to Claim 5 wherein the aqueous system is a cooling water system.

8. A method of controlling or inhibiting the growth and deposition of slime forming microorganisms in water which comprises adding to the water:

(i) A microbiocidally effective amount of an oxidant or oxidizing biocide for inhibiting the growth of microorganisms selected from the group consisting of

monopersulfates, perborates, peroxides and percarbonates, and

(ii) a microbiocidally effective amount of a microbiocide for inhibiting the growth of microorganisms selected from the group consisting of glutaraldehyde and the combination of glutaraldehyde, 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one.

9. A method of controlling or inhibiting the growth and deposition of slime forming microorganisms in water according to claim 2 wherein the oxidant is selected from the group consisting of monopersulfates, perborates, peroxides and percarbonates, and the microbiocide is glutaraldehyde.

10. The method of Claim 8, comprising a ratio of at least 10 parts of the microbiocide and 90 parts by weight of the oxidant to 90 parts by weight of the microbiocide and 10 parts by weight of the oxidant.

11. A microbiocidal composition comprising:

(i) a microbiocidally effective amount of an oxidant or oxidizing biocide for inhibiting the growth of microorganisms selected from the group consisting of monopersulfates, halogens, halogen releasing compounds, perborates, peroxides, percarbonates, perorganic acid, chlorine dioxide, persulfates, diperoxydodecanoic acid, permanganates, ozone and mixtures thereof; and

(ii) a microbiocidally effective amount of a microbiocide for inhibiting the growth of microorganisms selected from the group consisting of glutaraldehyde, limonene, bis(trichloromethyl) sulfone, 2-(decylthio)-ethanamine, dodecylguanidine hydrochloride, 2-(2-bromo-2-nitroethyl) furan, poly(oxyethylene (dimethyliminio)

ethylene (dimethyliminio) ethylene dichloride), alkyl dimethyl benzyl ammonium chloride, alkylamidopropyl propylene glycol dimethyl ammonium chloride phosphate, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, tetrakis-hydroxylmethyl phosphonium sulfate, tributyltetradecyl phosphonium chloride, 2-bromo-2-nitropropane-1,3-diol, 2,2-dibromo-2-nitroethanol, and sanguinaria extract; and

(iii) a surfactant/dispersant.

12. The composition of Claim 11, comprising:

(i) at least 0.1 ppm of said oxidant

(ii) at least 0.1 ppm of said microbiocide, and

(iii) about 5 ppm of said surfactant/dispersant.

13. The composition of Claim 11, comprising 0.5 ppm to 45 ppm of said oxidant and 0.5 ppm to 45 ppm of said microbiocide.

14. The composition of Claim 11, wherein the surfactant is a fluorinated surfactant.

15. The composition of Claim 11, further comprising an anti-corrosive material.

16. The composition of Claim 11, further comprising an anti-scale material.

17. The composition of Claim 15, wherein the anti-corrosive material is tetrapotassium pyrophosphate or sodium tripolyphospate.

18. The composition of Claim 16, wherein the anti-scale material is sodium polyacrylate.

19. The composition of Claim 1, wherein the oxidant is selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and the microbiocide is glutaraldehyde.

20. The microbiocidal combination of materials comprising at least 10 parts by weight of microbiocide and 90 parts by weight of oxidant to 90 parts by weight of microbiocide to 10 parts by weight of oxidant, wherein:

(i) said oxidant is selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and

(ii) said microbiocide is selected from the group consisting of glutaraldehyde and the combination of glutaraldehyde, 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one.

21. A microbiocidal combination of materials comprising at least 10 parts by weight of microbiocide and 90 parts by weight of oxidant to 90 parts by weight of microbiocide to 10 parts by weight of oxidant, wherein:

(i) said oxidant is selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and

(ii) said microbiocide is selected from the group consisting of glutaraldehyde.

INTERNATIONAL SEARCH REPORT

International application No. PCT/US95/13947

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/28, 29, 36, 37; 424/600, 613-616, 657, 660- 665, 667, 673, 677, 703, 709, 715, 723; 514/372, 557, 558, 559, 560, 705.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 4,802,994 (MOUCHE ET AL.) 07 February 1989, column 1, lines 48-52, column 2, lines 9, 45-51, 65-68, columns 3-4 and claims 1, 3 and 4.	1,2,8-10,19,21 ------------ 1-21
Y	US, A, 4,975,109 (FRIEDMAN JR. ET AL.) 04 December 1990, column 1, linrd 5-36.	1-21
P, X ----- P, Y	US, A, 5,368,749 (LA ZONBY) 29 November 1994, Examples 1 and 2 at columns 4-5, claims 1-3.	1-3, 5-6, 8-10, 19, 21 ----------- 1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search: 03 FEBRUARY 1996

Date of mailing of the international search report: 13 FEB 1996

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13947

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13947

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61L 2/00, 2/16, 2/18; A01N 35/00, 37/00, 37/02, 43/80, 59/00, 59/02, 59/08, 59/10, 59/12, 59/14.

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/28, 29, 36, 37; 424/600, 613-616, 657, 660- 665, 667, 673, 677, 703, 709, 715, 723; 514/372, 557, 558, 559, 560, 705.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I.Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) glutaraldehyde alone or in combination with isothiazolone compounds, claims 1-21.
II.Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) limonene or bis(trichloromethyl)sulfone, claims 1-7 and 11-18.
III. Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) 2-(decylthio)-ethanamine, claims 1-7 and 11-18.
IV.Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) dodecylguanidine hydrochloride, claims 1-7 and 11-18.
V.Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) 2-(2-bromo-2-nitroethyl)furan, claims 1-7 and 11-18.
VI.Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) poly(oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride), claims 1-7 and 11-18.
VII. Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) alkyl dimethyl benzyl ammonium chloride, claims 1-7 and 11-18.
VIII. Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) alkylamidopropyl propylene glycol dimethyl ammonium chloride phosphate, claims 1-7 and 11-18.
IX.Microbiocidal composition and method comprisingutilizing (i) an oxidant, and (ii) 2,4,4'-trichloro-2'-hydroxydiphenyl ether,claims 1-7 and 11-18.
X.Microbiocidal composition and methodcomprising utilizing (i) an oxidant, and (ii) tetrakis-hydroxylmethyl phosphonium sulfate, claims 1-7 and 11-18.
XI.Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) tributyltetradecyl phosphonium chloride, claims 1-7 and 11-18.
XII. Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) 2-bromo-2-nitropropane-1,3-diol or 2,2-dibromo-2-nitroethanol, claims 1-7 and 11-18.
XIII. Microbiocidal composition and method comprising utilizing (i) an oxidant, and (ii) sanguinaria extract, claims 1-7 and 11-18.
The thirteen inventions as set forth above each contain ingredient "(ii)," which are member(s) of structurally diverse class of compounds. The compounds of ingredient (ii) have no common structure, and belong to diverse class of compounds. Thus, the inventions are not so linked by a special technical feature as to form a single general inventive concept. See MPEP Appendix AI, Annex B, Example 23. The claims thus lack unity of invention under PCT Rule 13 and 37 CFR 1.475.

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